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Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14

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ABSTRACT Myeloid cell activation by lipopolysaccharides (LPS) involves two proteins, plasma LPS-binding protein (LBP) and cell-membrane CD14. Cell membrane CD14, anchored by a glycerophosphatidylinositol tail, is the cellular receptor for LPS–LBP complexes. Another form of CD14, without the lipid tail, circulates as a soluble plasma protein. In this work we show that soluble CD14 (sCD14) is required for activation of endothelial and epithelial cells by LPS. We propose that LPS–LBP complexes transfer LPS to sCD14, and the LPS–sCD14 complexes then bind to a cellular receptor. Support for this pathway comes from experiments in which LBP and CD14 in normal human serum are blocked by specific antibodies, experiments in which serum is replaced by purified LBP and sCD14, and experiments in which specific binding of [^{3}H]LPS to epithelial cells is quantitated.

Exposure to endotoxin (lipopolysaccharide, LPS) during Gram-negative sepsis results in the release of numerous inflammatory mediators. Cells that release these mediators include monocytes/macrophages (MΦ) and granulocytes. Two proteins are of principal importance in stimulation of these myeloid cells: LPS-binding protein (LBP), a plasma protein (1, 2), and CD14, a MΦ and granulocyte membrane protein (3). LBP is a 60-kDa glycoprotein found in normal human serum (NHS) at ≈10 μg/ml (4). It binds to LPS via the lipid A moiety with an affinity of $=10^9$ M^{-1} (5). CD14 is a 55-kDa glycoprotein anchored to the MΦ membrane via a glycerophosphatidylinositol anchor (6). The membrane-bound CD14 (mCD14) serves as a receptor for complexes of LPS and LBP (7). Current evidence supports the contention that the LBP/CD14-dependent pathway contributes to MΦ and granulocyte stimulation by LPS under physiologic conditions. Interestingly, a soluble form of CD14 (sCD14) lacking the glycerophosphatidylinositol anchor is also present in serum; its origin and function have not been defined (8).

Endothelial and epithelial cells may also play important roles in host responses to LPS during sepsis. Two distinct pathways for LPS stimulation of these cell types may occur by either direct stimulation by LPS or indirect stimulation via cytokines released from LPS-stimulated myeloid cells (9–14). Evidence for both pathways has been provided from *in vitro* studies with primary cell cultures and cell lines. Little is understood about the mechanisms that control LPS recognition and signaling by endothelial and epithelial cells. Because these cells are not known to express mCD14, there was no reason to consider that LBP and/or CD14 would be involved in regulating responses to LPS. However, LPS stimulation of endothelial cells has been reported to be enhanced by serum, and recently it has been shown that anti-CD14 monoclonal antibodies (mAbs) block effects of LPS on bovine endothelial cells cultured in the presence of serum (15). No comparable data are available for epithelial cells.

In this report it is shown that several epithelial-like cell lines, like endothelial cells, require serum for LPS stimulation of cytokine release. Importantly, evidence is provided for a specific mechanism of LPS stimulation of endothelial and epithelial cells that involves LBP and sCD14 and provides an explanation for the serum requirement displayed by both cell types. These data suggest that LBP and sCD14 in blood or in extravascular fluids may contribute to the consequences of endotoxemia by enabling LPS stimulation of endothelial and epithelial cells.

MATERIAL AND METHODS

Cell Sources. Human umbilical vein endothelial cells (HUVEC) were obtained as described (16) and maintained using 199 medium (Whittaker Bioproducts)/20% fetal bovine serum (HyClone)/heparin at 90 μg/ml (Sigma)/endothelial cell growth supplement at 30 μg/ml (Upstate Biotechnology, Lake Placid, NY). Identification of the endothelial origin was confirmed by morphology and staining with a fluorescent anti-von Willebrand factor antibody (The Binding Site, San Diego). SW620 and HT29 cells (human colonic adenocarcinoma cell lines) were purchased from the American Type Culture Collection (ATCC) (CCL 227 and HTB 38, respectively) and maintained with Dulbecco's modified Eagle's medium (DMEM; Whittaker Bioproducts)/10% fetal bovine serum (HyClone). Medium 199 and DMEM were supplemented with penicillin at 100 units/ml, streptomycin at 100 μg/ml, 10 mM Hepes, and 20 mM L-glutamine (all from GIBCO). Human microvascular brain endothelial cells (HBEC) were provided by Jay A. Nelson (The Research Institute of Scripps Clinic, La Jolla, CA) and maintained as HUVEC, replacing the fetal bovine serum by 10% NHS (Sigma).

Reagents. The LPSs used were the *Salmonella minnesota* wild-type LPS (List Biological Laboratories, Campbell, CA) and Re595 LPS (17). NHS came from a healthy donor and was heated at 56°C for 30 min. For LBP depletion, NHS was incubated for 16 hr at 4°C with a protein G-purified IgG

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Abbreviations: LPS, lipopolysaccharide; LBP, lipopolysaccharide-binding protein; HUVEC, human umbilical vein endothelial cells; HBEC, human microvascular brain endothelial cells; NHS, normal human serum; HSA, human serum albumin; VCAM-1, vascular-cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; mAb, monoclonal antibody; IL, interleukin; MΦ, monocyte(s)/macrophage(s); mCD14, membrane-bound CD14; sCD14, soluble-CD14; ASD, 2-(*p*-azidosalicylamido)ethyl 1,3'-dithiopropionyl.

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preparation at 10 mg/ml from a goat anti-human recombinant LBP antiserum or with nonimmune goat IgG at 10 mg/ml as control. Precipitates were removed by centrifugation. mAb 28C5 specific for CD14 and mAbs 1E8 and 18G4 specific for LBP were isolated from hybridoma culture supernate by using the MAPS II kit (Bio-Rad); mAb IB4 specific for CD18 was from K. Arfors, La Jolla, CA. Rabbit LBP was purified from acute-phase rabbit serum (1). Human sCD14 was immunopurified from culture supernates of CHO cells transfected with a plasmid encoding human CD14, using immobilized mAb 63D3. Human recombinant interleukin (IL)-1β was from J. M. Dayer, Geneva. The RPMI 1640 medium, NHS, human serum albumin (HSA; Miles), and LBP were found to be free of endotoxin by the limulus lysate assay (Whittaker Bioproducts). The sCD14 stock solution (1 mg/ml) used had endotoxin at 5 units per ml, corresponding to *S. minnesota* wild-type LPS at ≈1 ng/ml.

Cell Activation Experiments. Microtiter plates (Costar) were precoated with 0.1% gelatin (Sigma) in phosphate-buffered saline (PBS), pH 7.4 (Dulbecco's PBS; Irvine Scientific) for 15 min at room temperature. One confluent 75-cm² flask of third- or fourth-passage HUVEC was trypsinized with 1.5 ml of trypsin-EDTA (GIBCO), seeded into three 96-well gelatin-coated plates (200 μl of cell suspension per well), and grown to confluency in 2–3 days. The HBEC were treated the same as the HUVEC. Individual confluent 75-cm² flasks of SW620 cells or HT29 cells were trypsinized, as were the HUVEC, and the cells were seeded into two 96-well plates without gelatin coating, achieving confluency within 2 days. Before use, confluent cells were washed three times with RPMI 1640 medium/HSA at 1 mg/ml. Incubation of cells with LPS alone, with LPS in the presence of 2% NHS with or without additional antibodies, or with LPS in the presence of purified LBP or CD14 with or without added antibodies was done in RPMI 1640 medium plus HSA at 1, 2, or 20 mg/ml, respectively. Control experiments showed that the addition of HSA at 1, 2, or 20 mg/ml had no differential effect on the outcome of the experiment. All wells received premixed incubation medium at 200 μl per well, were done at 37°C in 5% CO_2/100% humidity, and were done, at least, in triplicate. Cell viability after incubations was tested (18).

Vascular-Cell Adhesion Molecule 1 (VCAM-1), Intercellular Adhesion Molecule 1 (ICAM-1), and Cytokine Assays. After an activation experiment culture supernates were saved at −20°C for cytokine determinations. Cellular expression of VCAM-1 and ICAM-1 was assessed with VCAM-1 or ICAM-1 mAb (R&D Systems, Minneapolis) and peroxidase-conjugated goat anti-mouse IgG (Cappel Laboratories). IL-6 and IL-8 levels were determined in supernatants from HUVEC, HBEC, SW620, and HT29 cells using commercially available sandwich ELISA kits (R&D Systems).

Binding of [³H]LPS to SW620 Epithelial Cells. The [³H]LPS was from R. Munford (University of Texas, Dallas) and used as described (19). SW620 cells were grown to confluency in 6-well plates (NUNC), yielding 7–10 × 10⁶ cells per well. The cells were washed three times with RPMI 1640 medium/HSA at 1 mg/ml at 4°C, after which they were incubated in RPMI 1640 medium/HSA at 2 mg/ml at 37°C for 1 hr, with [³H]LPS in RPMI 1640 medium/HSA at 20 mg/ml or with [³H]LPS/RPMI 1640/2% NHS/HSA at 1 mg/ml, and with mAbs 28C5 or IB4 at 10 μg/ml. After incubation the cells were chilled to 4°C, washed once with RPMI 1640 medium/HSA at 1 mg/ml, twice with PBS, and collected for counting. Results are expressed as pg of [³H]LPS bound per well, using 5.8 × 10⁶ dpm/μg as the specific activity. "No-cell" control wells were prepared by addition of DMEM/10% fetal bovine serum to wells for several days before treatment exactly as the experimental wells.

Cross-Linking of LPS to Serum Proteins. Re595 LPS was derivatized (20) and labeled with ^{125}I (21) to produce ^{125}I-labeled ASD [2-(*p*-azidosalicylamido)ethyl 1,3'-dithiopropionyl] LPS. The ^{125}I-ASD-LPS was incubated at 500 ng/ml with NHS for 3 min at 37°C and photolysed at 253 nm for 2 min on ice. Human LBP and sCD14 were immunoprecipitated with mAb at 50 μg/ml (1E8 and 18G4 for LBP; 63D3 and 28C5 for CD14) as first antibodies, and using a rabbit anti-mouse IgG at 250 μg/ml (Zymed Laboratories) as second antibody both for 3 hr at 4°C. Labeling of purified sCD14 by ^{125}I-ASD-LPS was studied in a 100-μl reaction mixture by incubating ^{125}I-ASD-LPS at 20 ng/ml with 4.5×10^{-9} M sCD14 in PBS, pH 7.4, at room temperature with or without 5×10^{-10} M purified rabbit LBP. At various times samples were withdrawn and photolysed on ice for 2 min. Labeled serum, immunoprecipitates, and proteins were analyzed by using SDS/PAGE on a 10–15% gradient gel and autoradiography. After development of the gel, labeled bands were excised to quantitate the bound ^{125}I.

RESULTS

Activation of Endothelial Cells by LPS. HUVEC cultured with LPS for 6 hr in RPMI 1640 medium/HSA failed to secrete IL-8 or IL-6 and did not express ICAM-1. In contrast, HUVEC cultured with LPS/2% NHS released IL-6 and IL-8 and expressed ICAM-1 (Fig. 1 *A–C*). Serum concentrations >2% did not support higher responses, and a serum concentration of 0.03% supported only minimal responses to LPS (data not shown). Because the NHS contains LBP and sCD14, two approaches were used to determine whether these proteins mediate HUVEC stimulation by LPS. The effects of anti-CD14 or anti-LBP were examined first. When mAb 28C5, an anti-CD14, was included in the incubation with LPS/2% NHS, the ICAM-1, IL-6, and IL-8 responses were all abolished. In contrast, the addition of mAb IB4, an anti-CD18, did not change the cell responses, indicating the specificity of the anti-CD14 effect. In experiments not shown, activation of HUVEC by IL-1β at 0.1 or 1.0 ng/ml in 2% NHS was not inhibited by mAb 28C5. A panel of 21 other anti-CD14 mAbs were tested for their abilities to inhibit HUVEC responses to LPS; MY4 (Coulter), 18E12, and 28C5 (R. W. Johnson Pharmaceutical Research Institute, La Jolla, CA), LoMo-1 (Zymed Laboratories), 3C10 (W. Van Vorhees, University of Washington, Seattle), and Cris-6 (Biodesign International, Kennebunkport, ME) were inhibitors of LPS-induced HUVEC activation.

Because LBP is required for the binding of LPS to CD14 (3) immunodepletion of LBP from NHS was tested for its ability to block a HUVEC response to LPS/2% NHS. Immunodepletion of LBP from the NHS with goat polyclonal anti-(human LBP) IgG reduced the IL-8 response nearly to that seen in the absence of NHS, whereas normal goat IgG had no effect (Fig. 1*D*).

To further test the role of CD14 and LBP in responses of HUVEC to LPS, serum-free medium was reconstituted with sCD14, LBP, or both (see Fig. 2). As shown in Fig. 2*A*, VCAM-1 expression in response to LPS in serum-free medium alone is barely observable. VCAM-1 expression was not recovered with rabbit LBP at 1 μg/ml. However, sCD14 at 2 μg/ml did reconstitute the response. A mixture of rabbit LBP at 1 μg/ml with human sCD14 at 2 μg/ml gave the same response as that seen with sCD14 alone (data not shown). As with the HUVEC response to LPS/2% NHS, the HUVEC response to LPS in the presence of sCD14 was abolished by mAb 28C5 but not by mAb IB4. Essentially identical results were obtained with HBEC (data not shown). The results of a more stringent examination of the requirement for LBP in this system are shown in Fig. 2 *B* and *C*. In the absence of serum, no IL-8 was secreted over 6 hr in response to LPS at 0.15 or 0.5 ng/ml. Addition of human sCD14 at 100 ng/ml resulted in a modest IL-8 response, and the addition of LBP

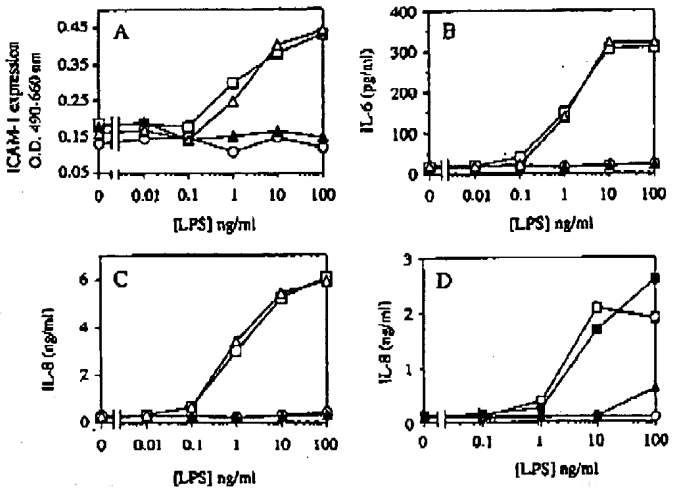

FIG. 1. Activation of HUVEC by *S. minnesota* wild-type LPS. (*A*) ICAM-1. (*B*) IL-6. (*C*) IL-8. ○, Cells incubated with *S. minnesota* wild-type LPS/RPMI 1640 medium; □, LPS/2% NHS/RPMI 1640 medium; ▲, LPS/2% NHS/anti-CD14 mAb 28C5 at 5 μg/ml; △, LPS/2% NHS/anti-CD18 mAb IB4 at 5 μg/ml. Incubation time of assay was 6 hr. (*D*) Secretion of IL-8 after 6-hr incubation with various LPS doses. ○, LPS/RPMI 1640 medium; □, LPS/2% NHS/RPMI 1640 medium; ▲, LPS/2% LBP-depleted NHS/RPMI 1640 medium; ■, LPS/2% mock-depleted NHS.

at 100 ng/ml and CD14 together heightened IL-8 release. These data suggest that the presence of LBP is probably important in LPS dose ranges found during endotoxemia (22).

Activation of Epithelial Cells by LPS. To determine whether LPS stimulation of epithelial cells displayed a similar dependence upon serum, sCD14, and LBP, a human epithelial-like cell line, SW620, was used. Like HUVEC the SW620 cells release IL-8 after exposure to LPS in the presence of NHS (see Fig. 3). Fig. 3*A* shows that LPS alone is not stimulatory for these cells, but addition of 2% NHS enables a good response to LPS at as little as 100 pg/ml. As with HUVEC, anti-CD14 mAb 28C5 blocked IL-8 secretion by SW620 cells, whereas anti-CD18 mAb IB4 had no effect. In Fig. 3*B*, the secretion of IL-8 is seen to be blocked by immunodepletion of LBP. As shown in Fig. 3*C*, purified sCD14 partially enabled secretion of IL-8, whereas purified rabbit LBP only minimally enabled IL-8 secretion. However, when both CD14 and LBP were present, IL-8 was secreted at levels seen with intact 2% NHS, and this response was sensitive to anti-CD14 but was not sensitive to anti-CD18. In kinetic experiments using LPS at 0.2 ng/ml or 1 ng/ml, with CD14 and LBP, the addition of LBP enhanced IL-8 secretion by the SW620 cells, as it did for HUVEC (data not shown). These data were reproducible with HT29 cells, another human epithelial cell line. However, A549 cells, a human cell line similar to pneumocyte type II cells, were not responsive to LPS (data not shown).

[^{3}H]LPS Binding to SW620 Cells. The functional evidence for an endothelial and epithelial cell receptor for LPS suggests that sCD14-dependent binding of LPS to these cells should be detectable. Because of the ease with which large numbers of the SW620 cells can be grown, these experiments were done with SW620 cells. Fig. 4 shows that binding of [^{3}H]LPS at 10 ng/ml to the cells was minimal in RPMI 1640 medium/HSA, but this binding was readily observable upon addition of 2% NHS. As with activation, binding was blocked by anti-CD14 mAb 28C5 but was not blocked by anti-CD18 mAb IB4. Thus, the specific binding, defined as the binding in the presence of 2% NHS that is blocked by mAb 28C5, is ≈125 pg per well at [^{3}H]LPS at 10 ng/ml. This quantity corresponds to 500 molecules per cell at 10 ng of [^{3}H]LPS per ml. A similar binding pattern was seen with concentrations of [^{3}H]LPS of 5, 3, and 1 ng/ml (data not shown). At 100 ng of [^{3}H]LPS per ml, no specific binding was detectable due to a high binding of [^{3}H]LPS in the "no-cell" control. Binding was maximal after 1 hr and could be observed at 37°C or 22°C but not at 10°C or 4°C (data not shown).

LPS Binding to CD14 and LBP. The data described above with HUVEC and SW620 cells imply that LPS binds to sCD14 and LBP in NHS. To demonstrate this directly, ^{125}I-ASD-LPS (500 ng/ml) was incubated for 3 min at 37°C in NHS and photolysed to crosslink the ^{125}I to whatever proteins had bound the LPS. Portions of the serum were then immunoprecipitated with anti-CD14 and anti-LBP antibodies, and the labeled serum as well as the immunoprecipitates

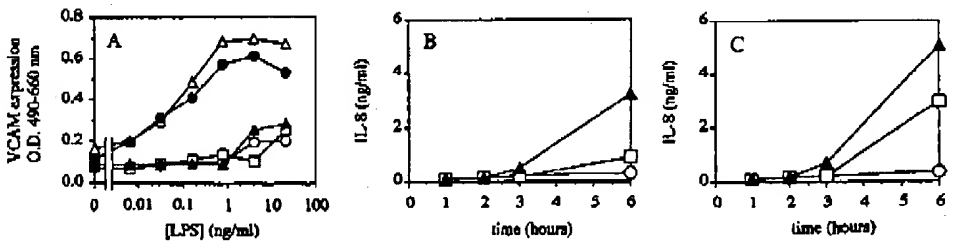

FIG. 2. Activation of HUVEC by *S. minnesota* wild-type LPS with purified plasma proteins. (*A*) VCAM-1 expression. ○, LPS/RPMI 1640 medium; □, LPS/purified rabbit LBP at 1 μg/ml/RPMI 1640 medium; △, LPS/purified human sCD14 at 2 μg/ml; ▲, LPS/sCD14 at 2 μg/ml/anti-CD14 mAb 28C5 at 20 μg/ml; ●, LPS/sCD14 at 2 μg/ml/anti-CD18 mAb IB4 at 20 μg/ml. (*B* and *C*) Time courses of IL-8 secretion by HUVEC. Secretion stimulated with LPS at 0.15 ng/ml (*B*) and LPS at 0.5 ng/ml/RPMI 1640 medium (*C*). ○, LPS alone; □, LPS/sCD14 at 100 ng/ml; ▲, LPS/sCD14 at 100 ng/ml/rabbit LBP at 100 ng/ml.

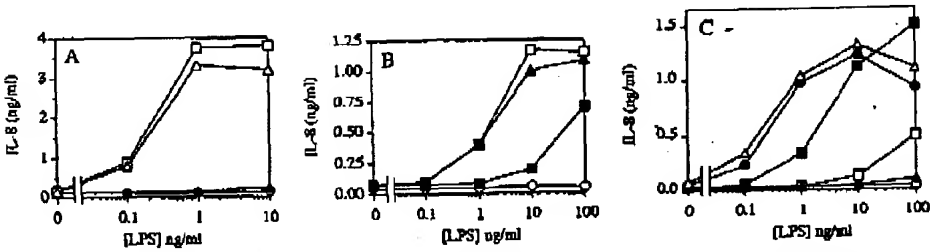

FIG. 3. IL-8 secretion by SW620 cells stimulated with *S. minnesota* wild-type LPS. (*A*) ○, LPS/RPMI 1640 medium; □, LPS/2% NHS/RPMI 1640 medium; ▲, LPS/2% NHS/anti-CD14 mAb 28C5 at 5 μg/ml; △, LPS/2% NHS/anti-CD18 mAb IB4 at 5 μg/ml. (*B*) LBP depletion of serum. ○, LPS/RPMI 1640 medium; □, LPS/2% NHS/RPMI 1640 medium; ■, LPS/2% LBP-depleted NHS; ▲, LPS/2% mock-depleted NHS. (*C*) Serum-free conditions. ○, LPS/RPMI 1640 medium/HSA at 20 mg/ml; □, LPS/LBP at 1 μg/ml/RPMI 1640 medium; ■, LPS/CD14 at 1 μg/ml; ●, LPS/CD14 at 1 μg/ml/LBP at 1 μg/ml; ▲, LPS/CD14 at 1 μg/ml/LBP at 1 μg/ml/mAb 28C5 (anti-CD14) at 10 μg/ml; △, LPS/CD14 at 1 μg/ml/LBP at 1 μg/ml/mAb IB4 (anti-CD18) at 1 μg/ml.

were analyzed by SDS/PAGE. Fig. 5 shows that the immunoprecipitates contain radiolabeled moieties with mobilities expected for LBP and sCD14. The labeled serum displays a single weakly labeled band of mobility intermediate between LBP and CD14. Examination of the Coomassie-stained gel (data not shown) shows that the high protein content of the serum sample severely distorts the electrophoretic mobilities just in the 55- to 75-kDa range.

The data described above also suggest that LBP should enhance the binding of LPS to sCD14, as shown in Fig. 6. ^{125}I-ASD-LPS was incubated with sCD14, with or without LBP, at a concentration equal to 1% that of the sCD14, before photolysis. Labeling of the sCD14 is hardly detectable in the absence of LBP even after 30 min, but in the presence of LBP, the sCD14 rapidly becomes labeled.

DISCUSSION

Our work suggests a mechanism to account for the serum-dependent stimulation of endothelial and epithelial cells by LPS. This mechanism, contrasted with what is known about LPS stimulation of MΦ, is depicted schematically in Fig. 7. LPS–LBP complexes may react in two ways: either, as shown previously, with MΦ mCD14 leading to MΦ activation, or alternatively, as shown in this work, with sCD14 to form sCD14–LPS complexes. The latter, in turn, react with endothelial and epithelial cells, leading to their activation. Previous reports have indicated a requirement (15, 23) for serum in endothelial cell responses to LPS and shown that anti-CD14 antibodies were inhibitory (15). Here we establish a functional role for sCD14 in the activation of endothelial cells and demonstration of an identical pathway mediating LPS-induced epithelial cell stimulation. In addition, we show CD14-dependent binding of [^{3}H]LPS to SW620 epithelial cells.

The individual steps in the activation pathway that we have described are all consistent with the known biochemistry of LPS, LBP, and CD14. That LPS binds to LBP in acute-phase rabbit serum is known (1); thus, the finding that LPS binds to LBP in NHS is largely confirmatory. Similarly, functional studies of MΦ activation by LPS strongly indicate that LPS binds to mCD14, and the direct interaction of ^{125}I-labeled ASD-Re595 LPS with CD14 on THP-1 cells has been observed (26). The data of Figs. 5 and 6 extend these results to sCD14, both in NHS and in purified form. Finally, the documented role of LBP in enabling LPS to bind to MΦ mCD14 suggests that LBP should also be able to foster LPS binding to sCD14, and this has been confirmed (24). Considerations such as these have led several authors to suggest that sCD14 might be important in MΦ responses to LPS *in vivo*

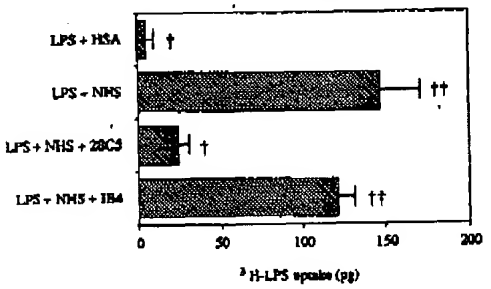

FIG. 4. Binding of [^{3}H]LPS (10 ng/ml) to SW620 cells in RPMI 1640 medium to which had been added 2% NHS, mAb 28C5 at 10 μg/ml, or mAb IB4 at 10 μg/ml, as indicated. Binding of 27 pg per well in the "no-cell" control well has been subtracted from the results (shown as means and SDs of triplicate determinations). Bars marked † and †† are significantly different at $P < 0.01$ by one-way ANOVA.

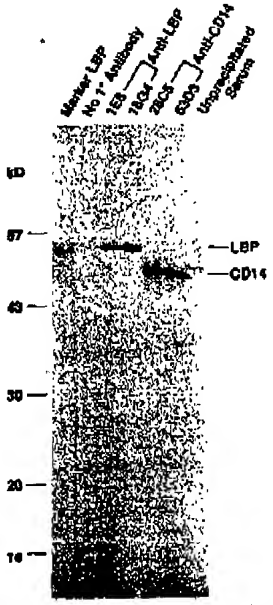

FIG. 5. Autoradiogram of LBP and CD14 labeled in NHS with ^{125}I-labeled ASD-Re595.

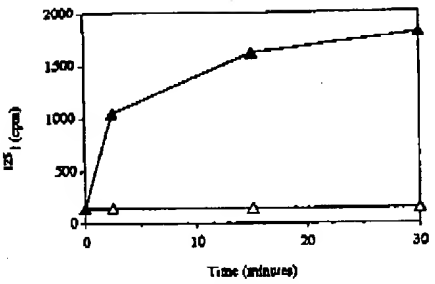

FIG. 6. Enhancement of the labeling of CD14 by LBP. △, Label incorporated into CD14 without LBP; ▲, label incorporated into CD14 with LBP.

(24, 25). Thus, it was unexpected that sCD14–LPS complexes should serve as agonists for endothelial and epithelial cells. There is no obvious precedent for this dual role of CD14 as receptor in one form and cofactor in another.

At first glance, the NHS immunodepletion data and the serum-free medium repletion data may seem partially contradictory. Thus, immunodepletion of LBP from 2% NHS blocks HUVEC activation despite the remaining presence of sCD14 in the NHS. However, consideration of the concentrations of sCD14 and LBP in NHS removes this apparent contradiction. sCD14 is present at 2–6 μg/ml (8, 24) in NHS. Thus, sCD14 should be present in the immunodepleted 2% NHS at 40–120 ng/ml. At this concentration of sCD14, the activation of the cells is strongly enhanced by LBP, as shown in Fig. 2 *B* and *C*. Furthermore, in NHS there are other proteins and lipoproteins among which LPS can partition. Thus, we would expect the dependency on LBP and CD14 to be more stringent in NHS than in serum-free medium fortified with either sCD14 or LBP. However, it is also possible that the requirement for LBP in association of LPS with sCD14 is less stringent than for association of LPS with MΦ mCD14.

The generality of these results is of some interest. Among endothelial cells we have tested HUVEC and HBEC with similar results. It seems likely that the bovine brain endothelial cells studied by Patrick *et al.* (15) also are responding to CD14–LPS complexes, and thus the pathway described here may be general for endothelial cells. Among the epithelial cells tested, the two colonic adenocarcinoma cell lines were responsive to CD14–LPS complexes. Primary cultures of epithelial cells have been shown to respond to endotoxin, but their dependency on CD14 was not tested (11).

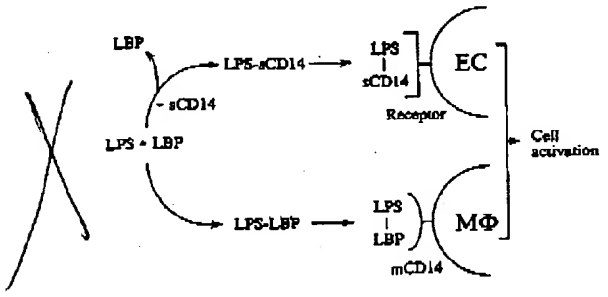

FIG. 7. Schematic diagram of the LPS-, LBP-, and CD14-dependent activation of MΦ or endothelial and epithelial cells (EC).

Obviously, the nature of the cellular receptor for the CD14–LPS complexes on the endothelial and epithelial cells is of considerable interest, although not addressed by these results. That there is a specific receptor for LPS on these cells is indicated by the data showing specific binding of [^{3}H]LPS to the SW620 cells. There is the possibility that endothelial and epithelial cells express very low levels of mCD14 and that the mCD14 acts as the cellular receptor for sCD14–LPS complexes. If so, the mCD14 on endothelial cells and MΦ have different properties with regard to LBP–LPS complexes because LBP opsonizes LPS-coated particles for MΦ but not for HUVEC (3). Similarly, LBP enables MΦ to respond to LPS in the absence of other proteins but does not enable either the HUVEC or SW620 cells to respond. Thus, our data suggest that the endothelial and epithelial cell receptor(s) for CD14–LPS complexes are distinct from receptor(s) for LPS–BP complexes.

Note Added in Proof. A functional role for sCD14 has been proposed (27).

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